FINAL REPORT FOR NOAA-COP PROJECT NO. NA16RG0521-01
R/COP-3

The project entitled "The Acute Toxicity and Bioaccumulation of Azinphosmethyl in Benthic Copepods..." commenced on November 1, 1991, as a cooperative effort between the U.S.C. Meiobenthic Toxicology Laboratory and the NMFS-Southeast Fisheries Science Center.
The purpose of this collaborative study was to characterize the acute and chronic toxicity of sediment-associated Azinphosmethyl to meiobenthic copepods, compare the relative importance of whole-sediment, aqueous and porewater exposure routes (and develop analytical methods for Azinphosmethyl measurement in these phases), and finally to measure Azinphosmethyl bioaccumulation and trophic-transfer amounts/effects through benthic copepods. It is well known that estuarine sediments sequester toxicants for months to years, yet methods are still severely limited for quantifying population-level toxicant effects on resident benthic infauna and subsequent risks of trophic transfer from benthos to higher trophic levels. To this end, we have exploited meiobenthos as a novel test group for sediment-associated pesticides and other more persistent xenobiotics. We have also studied other comparative species and exposure routes as part of this project, and those findings are also included in this report.

Facts about Azinphosmethyl

Originally registered in 1956, APM (O,O-dimethyl-s-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate; formula weight 317.3, tradename *Guthion;* **Figure 1**) is a potent acetyl cholinesterase (AChE) inhibitor which is widely used as an insecticide, acaricide, and molluscicide. APM may be applied by ground or aerial equipment at rates as high as 10.35 lb active ingredient per acre for field crops, tobacco and silviculture. APM is a non-polar OP with high sediment-binding affinity (K_{ow} = 360 at 20°C) and correspondingly low soil leaching potential. Its half-life in aerobic, non-sterile soils is 21 days; under anaerobic conditions the half-life increases three-fold to 68 days (EPA 1988). Water-solubilized APM is highly toxic to aquatic invertebrates and fish (LC_{50}'s of most species $\leq$ 100 $ug \cdot L^{-1}$ (EPA 1990)); but no published data exists for toxicity of sediment-bound and porewater APM residues nor for bioaccumulation rates/potential in non-target organisms (declared by EPA in 1990 as a major data gap along with acute toxicity profiles).

Similar to other organophosphate compounds, azinphosmethyl readily binds with AChE as its mode of action in eliciting toxicity. Azinphosmethyl is a derivative of thiophosphoric acid and as such is insoluble in water. However, upon conversion to its oxygen analog, a necessary step to inhibit AChE, azinphosmethyl becomes less lipophilic and a rapid hydrolysis occurs to result in less persistence (Murphy, 1986).

The most recent estimates have put azinphosmethyl use in the US at three million pounds active ingredients annually (EPA, 1986). From 1966 to 1985, 36 fish kills involving azinphosmethyl were documented in the US (EPA, 1986). In 1991 more than a dozen fish kills occurred in waters surrounding sugarcane fields in Louisiana after azinphosmethyl treatment. This number was reduced to three kills in 1992 after the achievement of stricter regulations (Chaillot, 1992). Fish kills have also occurred in coastal SC with water concentrations > 7 μg/L after runoff from tomato fields (Scott et al., 1990).

Azinphosmethyl toxicity to fish shows a wide range of LC50 values. Of five freshwater fish families tested by Macek and McAllister (1970), *Ictalurids* were the most tolerant with 96-h LC50 values as high as 3500 μg/L. Azinphosmethyl was the most toxic to the *Salmonidae* family with 96-h LC50 as low as 4.0 μg/L (Macek and McAllister, 1970). In the estuarine fish, *Fundulus heteroclitus*, laboratory tests revealed a 96-h LC50 of 32.16 μg/L with a no observable effect concentration of 4.95 μg/L (Fulton, 1989). In determining AChE inhibition in *F. heteroclitus* exposed to azinphosmethyl, Fulton (1989) found an estimated 24-h EC50 of 0.81 μg/L. In a life cycle toxicity test, sheepshead minnows were less tolerant with 78% dying after one week exposure to 2.0 μg/L azinphosmethyl. AChE activity was significantly inhibited in these fish at azinphosmethyl concentrations of 0.06 to 0.50 μg/L after 235 days exposure (Cripe et al., 1984).

Azinphosmethyl is generally more toxic to crustaceans than fish. In marine shrimp, reported LC50's ranged from 0.3 μg/L for *Crangon crangon* to 1.0 μg/L for *Pandalus montagui* in 48-h bioassays (Portmann and Wilson, 1971). Moore (1988) reported a 96-h LC50 of 0.93 μg/L in adult grass shrimp, *Palaemonetes pugio*. In 24-h bioassays, LC50 values ranged from 4.4 to 16.8 μg/L for the freshwater grass shrimp, *P. kadiakensis,* collected from pesticide contaminated sites. Shrimp collected from an uncontaminated site experienced an LC50 of 8.9 μg/L (Naqvi and Ferguson, 1970).

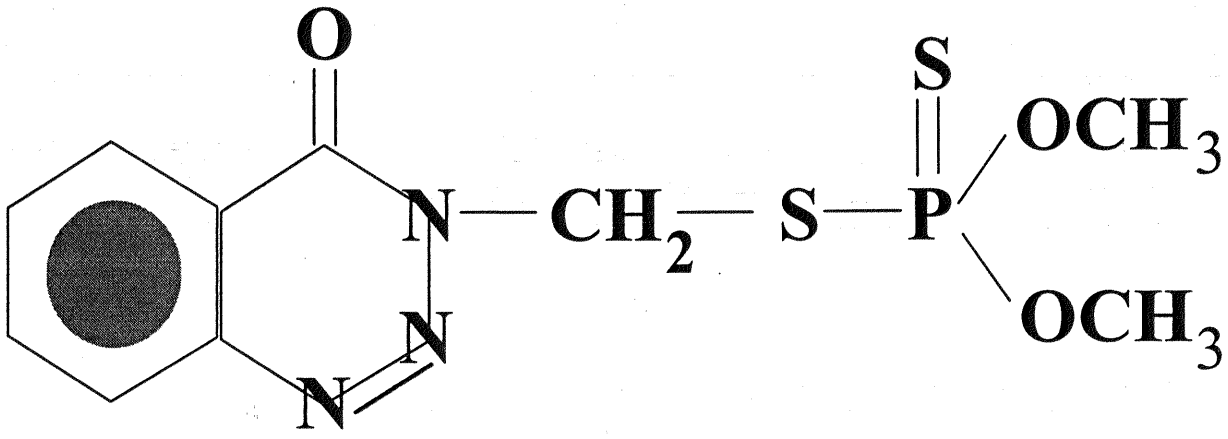
MEIOBENTHOS RESPONSES TO AZINPHOSMETHYL:

Research at the U.S.C. Meiobenthic Toxicology Laboratory and the NMFS Charleston Lab has centered upon (1) continuous high-density sediment culture of fully-acclimated stocks of the infaunal burrowing copepod *Amphiascus tenuiremis* for acute and chronic bioassays and trophic studies, (2) short-term culture of the sediment-interface copepod *Microarthridion littorale* for acute toxicity tests, (3) 96-h acute LC_{50} tests over NINE triplicated concentrations of APM-spiked muddy sediments for both species, and nine sediment-free aqueous APM solutions for *Amphiascus tenuiremis*, (4) sex-specific sensitivities/comparisons of *Amphiascus tenuiremis* to sediment-associated and aqueous APM, (5) sediment porewater 96-h acute toxicity tests with *Amphiascus tenuiremis* employing a novel 3-mL microassay using porewater collected from ultra-centrifuged sediments, (6) definitive modelling of APM copepod toxicity by our empirical data in comparison to the "Equilibrium Partitioning Approach to Sediment Quality Criteria" (DiToro *et al.* 1991), (7) chronic 14-day sublethal effects bioassays measuring reproductive output of virgin *Amphiascus tenuiremis* mated with males under APM exposure, (8) 96-h bioaccumulation/96-h depuration of ^{14}C-APM by *Amphiascus tenuiremis* and *Microarthridion littorale*, (9) 96-h to 26-day comparative toxicity tests with the more persistent and more extensively used organophosphate insecticide Chlorpyrifos, and (10) trophic transfer studies with APM using a sediment to copepod to fish predator model. **Figure 2** depicts several of the known pathways for meiofaunal predation by macrofauna.

Acute Toxicity of Sediment- and Porewater-Associated Azinphosmethyl on Benthic Copepods:

Our primary bioassay organism *Amphiascus tenuiremis* is a diosaccid harpacticoid copepod. Diosaccid copepods are the most abundant, diverse and widely-distributed family of sediment-dwelling copepods. Most species produce two egg sacs (hence the family name) containing 12

Azinphosmethyl (APM)



Structure of azinphosmethyl. Molecular weight 317, melting point 73° C, octanol:water partition coefficient 360 at 20° C, sorption coefficient 1000 ml/g at 25° C (Wauchope et al. 1992).